On the Development and Structure of the Trochophore of Hydroides uncinatus (Eupomatus).

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With Plates 21–23 and 29 Text-figures.

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1. Introduction.

While working at Naples some years ago, I was led to investigate the early development of the Annelid Eupomatus with a view to determining the origin of the mesoblast bands and their relation to the head-kidneys. This species is common at Naples and breeds throughout the year. The blastulæ and gastrulæ are very hardy, and development is normal under the adverse conditions of heat and impure sea-water incidental to their study under laboratory conditions. Fertilisation takes place quickly when the ripe generative

products are brought together, and material can be easily obtained of any stage. The trochophores can be readily reared to the adult worm in small jars of sea-water to which sufficient food is added from time to time, in the form of cultures of the common Diatom Nitzschia closterium. On this they rapidly grow, and soon attach themselves to the sides of the culture jars and form their tubes.

The minuteness of the egg is a serious disadvantage, however, in following the changes that lead up to the establishment of the trochophore. The fully formed larva barely measures 65 μ in diameter, and the pre-trochophoral stages are very small, and the cells of the blastulæ and gastrulæ are unusually minute. In following the origin and growth of the head-kidneys one is forced to depend almost wholly on sections, and sectioning larvæ of this size is a tedious proceeding.

In the Serpulid Pomatoceros I soon found a more suitable object in which to trace the development of the head-kidneys. The egg is larger and more deeply pigmented. In the arms of the "cross-cells" this pigment quickly becomes segregated on development, where it affords a ready means of orientation. For these reasons I early abandoned the study of Eupomatus for that of Pomatoceros, on which I hope shortly to complete my "Studies on the Development of Larval Nephridia," by publishing a full account of the origin and growth of these organs in this animal.

The present notes dealing with Eupomatus, although incomplete, I have thought worthy of publication, as they deal with the formation of the trochophore and the appearance of the cœlomesoblast. They derive some importance from the fact that on this Annelid, Hatschek (**17**) conducted his classical investigations on the development of the mesoblast bands—investigations which have played so prominent a part in all our speculations concerning the mesoderm. Any revision, therefore, of the subject on the same material as that studied by him is not without interest.

In the following account I have incorporated some drawings and notes of Hydroides pectinata, kindly placed at my disposal by Prof. E. B. Wilson, which I believe were made by him some years back.

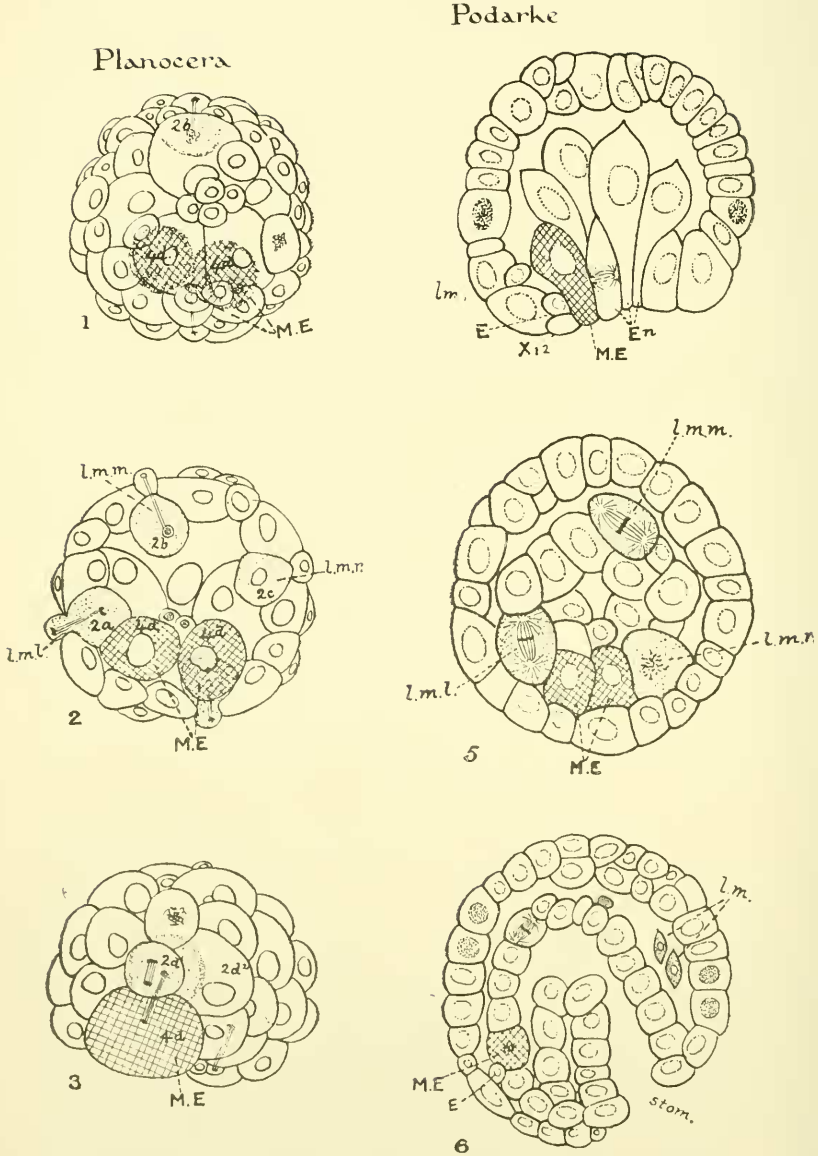
One object I have kept in view has been that of following the changes leading from the gastrula to the formation of the trochophore. In the numerous careful accounts of the development of Annelids that have been published few attempts exist to connect the cell regions of the early stages with the organs of the trochophore. Many of the early embryologists, as Kowalevsky (23), Agassiz (1), Hatschek (18) and Salensky (30) seem to start their studies only with the young larva, when the rudiments of the larval organs have already appeared. On the other hand, many of the more recent investigators, commencing with the unsegmented egg, frequently fail to carry their studies far enough when they stop short at the end of gastrulation, and before the definitive organs of the larva have appeared.

Some considerable confusion has arisen through taking the conditions found in relation with the mesoderm at relatively late stages, and considering these same relations to hold in the early phases. This is seen in the work of Hatschek (18) and Fraipont (12) on Polygordius; and has resulted in some error with regard to the head-kidney rudiments, larval and cœlomesoblast.

In all Annelids with a free-swimming larva such as that of Eupomatus there is always a considerable interval between the end of gastrulation and the assumption of the full trochophoral condition. This period, for the sake of convenience, I shall refer to as that of the pre-trochophoral stage. It is the period of which we know the least in the development of Annelids.

The excellent papers of Woltereck (52) on Polygordius and Torrey (41) on Thalassema have done much to advance our knowledge. The early cell-regions have here been traced clearly to their ultimate fate in the organs of the trochophore. Woltereck has shown that the head-kidneys arise early and

TEXT-FIGS. 1–6.

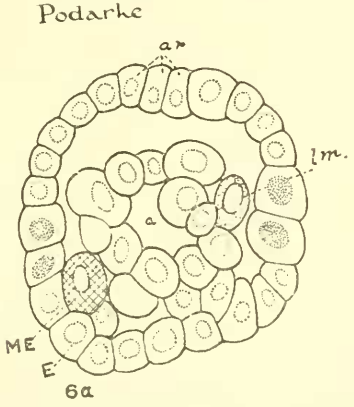


Text-figs. 1–3.—Early segmentation stages of Planocera (Surface). Text-figs. 4–6.—Podarke (Treadwell). *E.* 1*st* endoderm cell budded off from $4d_2$. *En.* Entomeres. *l.m.l.* Left portion of the ectomesoblast. *l.m.m.* Median portion of the same. *l.m.r.* Right portion of the same. M.E. Cœlomesoblast. *Stm.* Stomodæum. $X_{1,2}$. Anal cell.

before the mesoblast bands. They are already functional before the bands have appeared, for the pole-cells so conspicuous in Hatschek's figures have no existence at this stage.

It is true that Meyer (**27**), from the study of late stages, came to the conclusion that larval mesoblast was a structure apart from the cœlomesoblast, but I doubt if the evidence derived from the study of these late stages in Polygordius alone is sufficiently convincing.

TEXT-FIG. 6A.



Section through a late gastrula stage of Podarke (Treadwell). *a.* Archenteron. *a.r.* Apical rosette. E. First endoderm cell budded off from $4d_2$. *l.m.* Larval or ectomesoblast. ME. Cœlomesoblast.

Within the last twenty-five years a large literature has grown up with regard to the question of the mesoderm, and embryologists have held many opinions regarding its origin and significance. These conflicting views are roughly reducible, however, to two groups, each of which has been advocated with more or less success. To the first belong those who consider the mesenchyme (larval mesoblast, ectomesoblast) and mesothelium (definite mesoderm or cœlomesoblast) as one and the same structure; to the second belong those who consider them as two separate structures.

The first consider they have a common, while the second claim they have a separate origin.

Hatschek, as the result of his studies on Polygordius (**18**), Echiurus (**16**), Eupomatus, and Teredo (**17** and **15**), many years ago pointed out the difference between the irregular scattered cells of the mesenchyme and the definite cells of the mesoblast bands. He claimed, however, to have observed the origin of the mesenchyme cells from the mesoblast bands. In his opinion mesenchyme and mesothelium arise from a common foundation. This was followed by Wilson's (**48** and **47**) work on Hydroides, Polygordius and Lumbricus, where he found a complete gradation from the stellate cells of the mesenchyme scattered through the blastocœl to the round fixed cells of the anterior ends of the germ bands. Many other observers have pointed out more or less the same thing, as, for instance, Roule (**29**) in Enchytræoides, Fraipont (**12**) in Polygordius, Bürger (**5**) in Nephelis, Hirudo and Aulastoma, Hacker (**13**) in Polynoe. The common nature of both mesenchyme and mesoderm at one time gained wide acceptance through its adoption and elaboration by the brothers Hertwig (**19**) in their well-known 'Cœlomtheorie.'

On the other hand, the majority of those embryologists who have recently investigated the development of Annelids and Molluscs hold that these structures are both ontologically and phylogenetically distinct; that the mesenchyme has an origin apart from the cœlomesoblast, that it arises in a peculiar fashion from the ectoderm; hence they have sought to denote this in the name they have applied to it, i.e. that of ectomesoblast. The cœlomesoblast, on the contrary, is usually segregated in a single large cell seen in the ventral lip of the blastophore.

Kleinenbergh (**21**) was perhaps the first to lead the way towards this conception of the nature of mesoderm and mesenchyme, in his paper on the development of Lopadorhynchus, where he pointed out that the mesoderm arises as a membrane between the two primary layers, and, as he

thought, direct from the ventral side of the ectoderm. This was followed by the work of Whitman (**45**) on Clepsine, Bergh (**3**) on Lumbricus, Vejdovsky (**44**) on Oligochæts. Schimkewitsch (**32**) in Dinophilus described a separate origin of the mesenchyme in the anterior end of the larva from the definite mesoderm of the posterior region. Finally the separate nature of mesenchyme and cœlomesoblast has been most ably championed in the very extensive researches of Meyer (**27**) on the mesoderm of Annelids.

In the work of the cell-lineage investigators, however, the distinction between larval and cœlomesoblast has been most definitely brought to light. In all Annelids, Lamellibranchs, and Gasteropods studied by them, with one exception, the cœlomesoblast invariably arises from a large cell in the ventral side of the blastophore (*4d*). The one exception is the Annelid Capitella, where, according to Eisig (**11**), it arises from the third and fourth quadrants of the third quartette. Here the cell *4d* contains a little larval mesoblast, but the main portion contains ectoderm. In Molluscs, according to Conklin (**7**), *4d*, while containing the cœlomesoblast, is more than half endoderm. In the Annelid Podarke, according to Treadwell (**42**), *4d* divides and then sinks in, and takes up its position in the endoderm of the archenteron (Text-figs. 4, 5, 6). Here at a later stage it gives rise to the cœlomesoblast.

At the time *4d* is being invaginated, or even before, irregular ectoderm cells are given off into the interior of the blastocœl; these are the larval mesoblast cells. They migrate inwards and scatter throughout the cavity. Their origin has been determined in a large number of forms, first by Lillie (**25**) in Unio, and then by Conklin (**7**) in Crepidula, Treadwell (**42**) in Podarke (Text-fig. 5, *l.m.r.*, *l.m.l.*, *l.m.m.*), Wierzejski (**46**) in Physa (Text-fig. 11, *l.m.r.*, *l.m.l.*), Torrey (**41**) in Thalassema (Text-figs. 8 and 9, *l.m.r.*, *l.m.l.*, *l.m.m.*). The mode of origin of the ectomesoblast, therefore, is distinctly in opposition to that of the cœlomesoblast. In Unio it arises asymmetrically, and only afterwards takes

up a bilateral position. In Thalassema it arises from the first and third quartettes. In some thirty Annelids it can be said definitely that the cœlomesoblast arises from the posterior cell of the fourth quartette, while the larval mesoblast arises from the first and third. This title of "larval mesoblast" does not mean necessarily that it is confined alone to the organisation of the larva, for the greater part of it enters into the structure of the adult. The same has been shown to be the case in a number of other Annelids, as in Polygordius, Podarke, and Thalassema.

Meyer long ago put forward the theory that the mesenchyme of higher forms corresponds with the mesoderm of the lower; that the larval mesoblast of Annelids and Molluscs is to be homologised with the adult mesoderm of Platodes. Wilson (**50**) has shown in Leptoplana that the mesoderm in this Polyclad arises from the second and third quartettes, while in Annelids the larval mesoblast, as I have mentioned above, takes its origin from the same quartettes. He has established that here the large cell *4d* is almost entirely entoblastic. The early development of the Polyclad Planocera has been studied by Surface (**40**): "At the forty-four cell stage the posterior cell of the fourth quartette (*4d*) buds a single large cell into the interior of the embryo; both of these subsequently divide bilaterally (Text-fig. 3). Of these four cells the two upper and inner (Text-fig. 3, *2d*) give rise to a portion of the mesoderm, and possibly a small part of the endoderm (Text-figs. 1–3, *4d*). The lower pair lying on the surface of the embryo give rise practically to all the endodermal part of the alimentary canal." Thus the history of this cell (*4d*) in this Polyclad shows a remarkable resemblance to its homologue in Molluscs and Annelids. "A portion of the mesoderm, chiefly that part lying round the pharynx, is derived from cells of the second quartette, and thus corresponds with the secondary mesoblast or larval mesenchyme of Annelids and Molluscs (Text-fig. 1, *2b*). In the spiral cleavage the segregation of the ectoblast in three quartettes, the formation of a large portion of the mesoderm

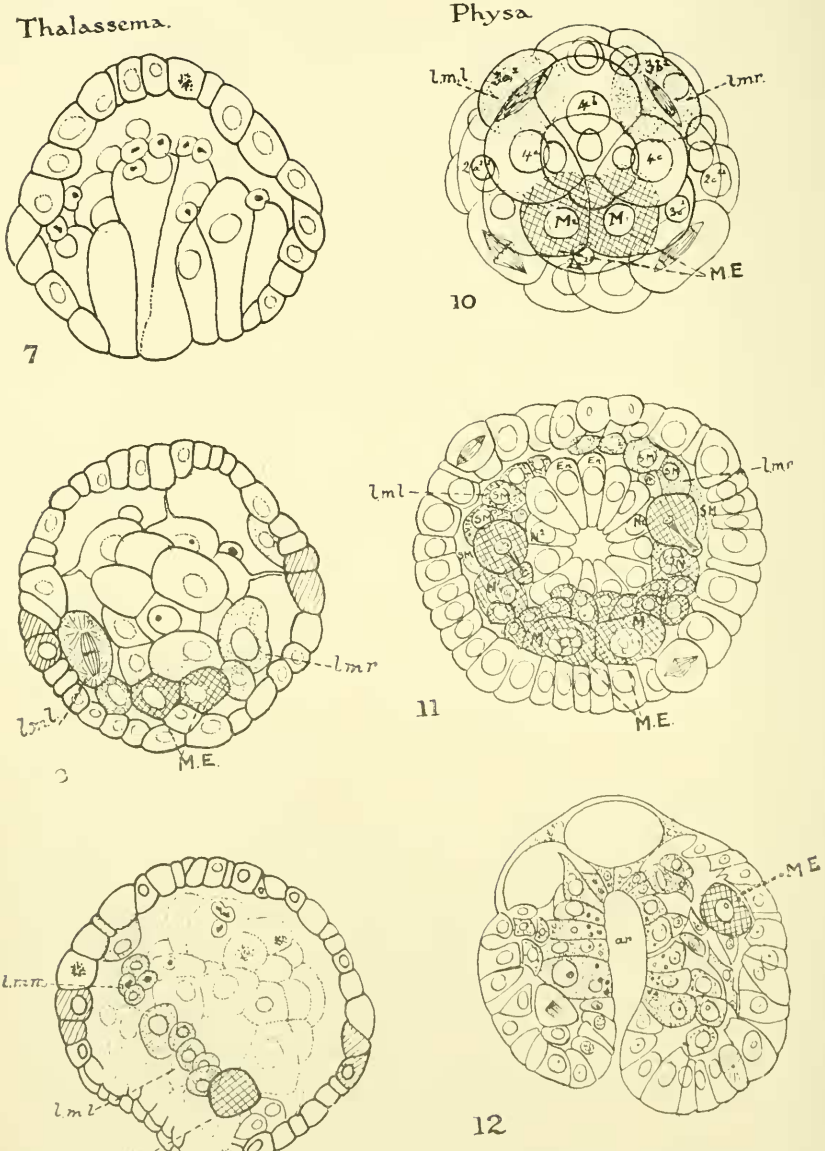
from *4d*, the formation of the apical cells, and in many other details, the development corresponds to that of Annelids and higher forms."

In Platodes the mesoderm has a radial origin, and this is also the case in a number of Annelids with regard to the larval mesoblast. I have also mentioned in a number of Annelids and Molluscs that a small portion of *4d* is entoblastic. The condition in Polyclads, where the greater part of *4d* is entoblastic, is suggestive of a more primitive condition than that found in Annelids. If the germ-cells in the Polyclad arise from the *4d* portion of the mesoderm, then the homology of this cell with the cell *4d* of Annelids would be complete. The history of the posterior cell of the fourth quartette in Polyclads, Annelids, Lamellibranchs, and Gastropods has a remarkable resemblance in all these forms, and the relation it shows with the endoderm of the gut points clearly, as Wilson (**50**) has said, to the way in which teloblasts have arisen by progressive specialisation from a purely endodermic origin of the cœlomesoblast as retained in an unaltered condition in the Echinodermata to-day. As he says, it is difficult to explain these facts otherwise than on the grounds "that cell outlines represent definite boundaries of differentiation areas in the developing embryo."

Child (**6**), on the contrary, claims that no importance can be attached to resemblances of this nature, and that in the case of the cell *4d* they are purely cœnogenetic, and have to do with the formation of the larval body from a growing region at its posterior extremity, and the resulting segregation of material at this point. I think this cannot be said of all cases where there is a similar segregation of the cœlomesoblast. The growth of the adult from the Glochidium larva is different in many respects from the growth of the adult worm from the trochophore, yet in both we get a marked segregation of the cœlomesoblast.

With the high degree of specialisation shown by eggs that give rise to a free-existing larva, the cœlomesoblast, which primitively arose as diverticula from the gut, became restricted

TEXT-FIGS. 7–12.



Text-figs. 7–9.—Gastrulation stages of Thalassema (Torrey).
Text-figs. 10–12.—Physa (Wierzejski). M.E. Cœlomesoblast. *l.m.l.* Left portion of the ectomesoblast. *l.m.m.* Median portion of same. *l.m.r.* Right portion of same.

in the course of time to certain cells in the wall of the archenteron, and as development became progressively specialised their origin became localised in the posterior cell of the fourth quartette.

It is from the wider and more definite facts of comparative anatomy rather than from those drawn from development alone that the real value of Meyer's theory lies. It is evident that the germ-cells—the foundation of the later cœlomesoblast—are much older in the phyletic sense, as Kleinenbergh (**21**) and Meyer (**27**) long ago pointed out, and as Eisig (**11**) has recently stated, than the two primary germ layers, and that they were differentiated long before the ectoderm and endoderm had been evolved as separate structures, as is the case to-day in Volvox. Meyer's theory has been recently considered by Lang (**24**) and Eisig (**11**) so exhaustively that it is unnecessary for me to go into it here. No theory certainly accounts for so many facts or has been so widely supported by evidence, both anatomical and embryological.

Thus the separation of the mesoderm into two definite portions is a characteristic feature of the development of Polyclads, Annelids, and Molluscs. It remains to be mentioned that in a few Molluscs a larval or ecto-mesoblast has not been observed or is apparently wanting. This would seem to be definitely the case in Aplysia, the late stages of which have recently been studied by Carr Saunders and Miss Poole (**31**). In Umbrella, Heymons (**20**) has been unable to find this structure, but he suggests that possibly in stages later than those he studied ectoderm cells may migrate into the interior of the larva and form mesoderm. In Neritina Blochmann (**4**) also fails to figure it; but both Heymons and Blochmann's work was done at a time when the importance of the larval mesoderm was hardly recognised, and ultimately it may prove to be present in these forms. Its absence in Aplysia, however, seems to be clearly established. It is hard to understand why this should be the case, as the majority of Molluscs possess a larval mesoblast, and one is present in Fiona.

Korschelt (**22**) has called attention to the relation of the ecto- and cœlomesoblast in Physa. If we take a section of such a stage of Physa as is shown in Text-fig. 11, he points out that the ecto- and cœlomesoblast between them form a complete ring round the blastopore. He thinks this condition points to the conclusion that in Annelids and Molluscs ecto- and cœlomesoblast were originally one structure, which has been divided and specialised as the result of larval development. In Phoronis and the other great group of animals of the Deuterostomia type this has not taken place. Phoronis is undoubtedly closely related to the Annelids in the Actinotrocha stage, with its solenocyte-bearing nephridia and ciliated rings, but shows no segregation of the cœlomesoblast into pole-cells.

From the work of De Selys Longchamps (**34**) we know that the mesoderm consists of a large number of irregular cells scattered throughout the blastocœl. I have shown (**36**), and it has also been clearly demonstrated by the work of other investigators, that these cells arise in the region of the blastopore, or from the line along which the blastopore has already closed. The cells resemble the larval mesenchyme of Annelids more than the cells of the Annelid cœlomesoblast.

In Brachiopods the mesoderm is also of the irregular variety, and arises from the cœlom, which is here a direct outgrowth from the anterior end of the primitive archenteron, as Conklin (**7**) has recently described in Terebratulina. No division into ecto- and cœlomesoblast can be distinguished, and it is purely cœlomesoblastic.

There would thus seem to be a sharp division between Phoronis and Brachiopods on the one hand, and Annelids and Molluscs on the other. In one we get a sharp division of the mesoderm into two portions, while in the other there is no such division. Korschelt (**22**) thinks that without a more definite knowledge of how the cœlomesoblast arose in the hypothetical Annelids, we cannot reconcile these two types of mesoderm formation.

It appears to me, however, that in Phoronis, or at least in the early stages of the Actinotrocha larva, we have exactly the same thing as in Annelids.

I have said that in Phoronis the mesoderm arises in the region of the blastopore as a number of irregular cells, which are budded off into the blastocœl. These scatter throughout the cavity, where they give rise to the mesodermic structures. According to De Selys Longchamps (**34**), some of these cells in the trunk region collect to form a rather imperfect cœlomic sac about the rectum or posterior portion of the stomach. I was of opinion, however (**36**), that the cells that gave rise to this sac had their origin in the gut wall, but of this I was by no means certain. In any case, in Phoronis we have the mesoderm showing a specialisation into a cœlomic portion, forming the primitive cœlomic sac, and the irregular mesodermal cells scattered throughout the blastocœl. Whether we regard the cœlomic portion as arising from the gut wall or not, it seems to me we have here the two forms of mesoderm as in Annelids, and that Phoronis is intermediate between Annelids and animals in which the mesoderm is entirely cœlomic. Korschelt (**22**) sums up the mesoderm formation under five heads, which are worth reviewing in this connection.

1st. Mesoderm band formation from teloblasts or pole-cells, as in Annelids and Molluscs.

2nd. Secondary mesoderm band formation, a modification of the above process, and re-multiplied in Arthropods and Cephalopods.

3rd. Formation of mesoderm from gut pouches.

4th. Formation of mesoderm from solid out-growths of the gut.

5th. The mesenchyme cells alone give rise to the cœlom and all the mesodermic structures.

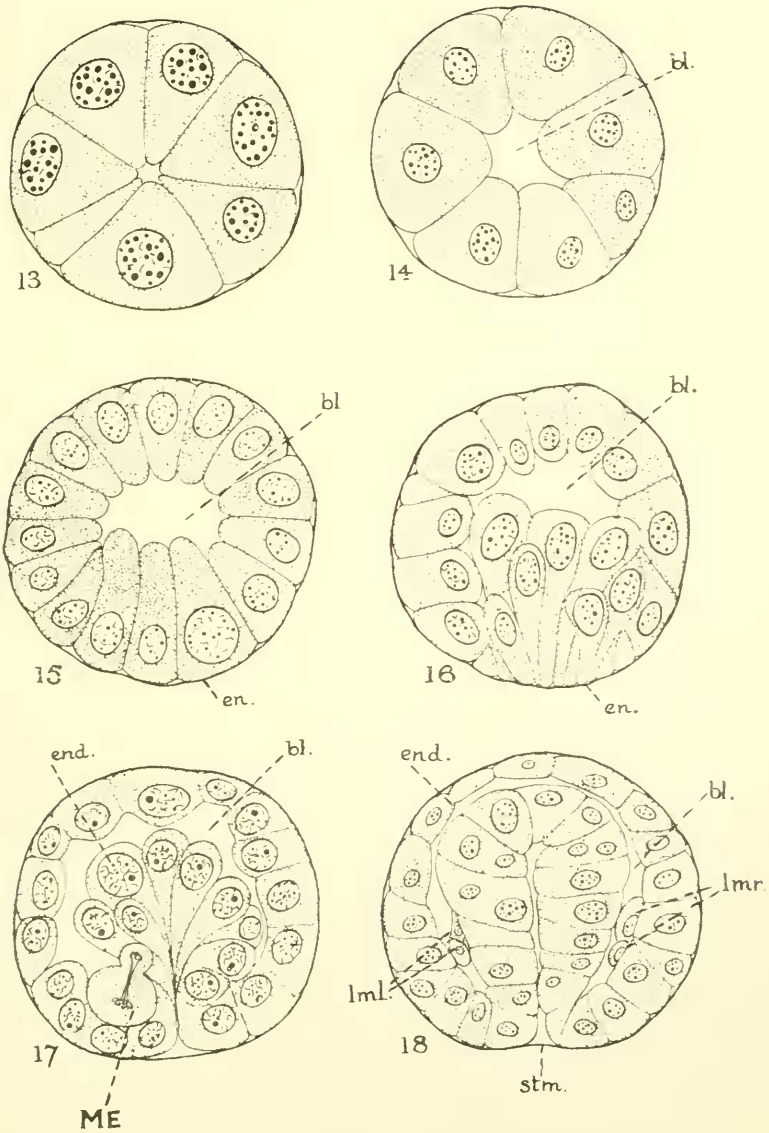
To the trochophore originally described by Hatschek (**17**) in Eupomatus undue importance has perhaps been attached, for such a trochophore is possessed by only a limited number of Annelids, and is almost exclusively confined to the group

of the Serpulids. Our text-books frequently cite it as a typical trochophore, although most Annelids possess a trochophore quite different. The trochophore characteristic of the majority of Polychæts is one such as that of Sabella or Nereis, and not that of the Serpulids. This possesses no head-kidney, and the mesoderm bands develop under conditions that modify their growth as compared with those of Eupomatus. The blastocœlic cavity in these is always greatly reduced or entirely obliterated, and gastrulation is usually epibolic; while in the Serpulid larvæ there is always a large blastocœlic cavity, and gastrulation is by invagination. The egg in the majority of the Serpulids, again, is small and contains very little yolk, although forms like Spirorbis and Sabella contain a considerable quantity. It is hard to make any fast distinctions, however, for larvæ occur in the same family, and even in the same genus, which differ entirely in this respect. The principal cause of this great diversity of form is due in most cases to the modification undergone by their locomotor organs, as the result of their adoption of different life-habits. Frequently closely related larval forms differ greatly in this respect. If they live a free-swimming pelagic existence, or the contrary, their locomotor organs are correspondingly developed or reduced. Terebella conchilega, leading a pelagic life, possesses strongly developed ciliated rings, and is a powerful swimmer, while Terebella meckelii, for the most part spending its larval existence in the jelly-like mass in which the eggs are deposited, is uniformly ciliated, and lacks these structures.

Thus the tuberculous Polychæts can be divided roughly into two classes on the basis of their possession or non-possession of a trochophoral stage. The first of these, including Eupomatus, Pomatoceros, and Psygmobranchus, possess typical free-swimming larvæ with well-developed prototroch and ciliated rings; while a second group, including some of the Terebellids, Aricia and Arenicola, do not possess a free-swimming stage, are often uniformly ciliated, and are poor swimmers. In addition, we

TEXT-FIGS. 13-18.

Eupomatus



Sections of segmentation and gastrulation stages of Eupomatus. *bl.* Blastocœl. *end.* Endoderm. ME. Cœlomesoblast. *lml.* Left portion of ectomesoblast. *lmr.* Right portion of same.

find a very large number of errant forms, which have undergone so much modification that in many cases it is difficult to say to which group they belong. In the first class of the pelagic type we have the larvæ of Nereis, Phyllodoce, and Aphrodite, while in the second we have forms like Diopatra, Ophryotrocha, and many of the Eunicid larvæ. Probably the most difficult to class of all are these last, on account of their great variation (Häcker, **13**).

Without some knowledge, therefore, of the mode of life of the primitive ancestors of the Polychæts, and the conditions under which they existed, it is difficult to decide which of these various larval types is the most primitive. It is probable that the free-swimming type has been recently evolved, and is a more highly modified one than the uniformly ciliated type, that the trochal has been derived from the atrochal form. And this is borne out by the fact that in its most perfect form, as in Eupomatus, it is found in so relatively few Annelids.

2. Review of Literature.

The early development of the Serpulid Annelids has been studied by a number of investigators. The earliest account is that of Stossich (**39**) in 1878, who described in some detail the development of Serpula uncinatus (Eupomatus) and S. glomerata. It is clear from his figures that many of his larvæ were abnormal. I have obtained many similar larvæ during the hot months in Naples, when the temperature of the Laboratory sea-water was unusually high. Through the study of these larvæ Stossich came to many erroneous conclusions.

Salensky (**30**), in 1883, studied the development of Psygmobranchus and Terebella. In these forms the presence of a considerable quantity of yolk and the absence of a true trochophoral stage considerably modify the course of development. He arrived at no certain conclusions regarding the origin of the mesoderm, although he observed the teloblast cells of the mesoderm bands.

Conn (**9**) pointed out that in Serpula the egg-chorion is never thrown off, but remains as the cuticle of the larva. The gastrula has three noticeable features. The blastopore is not round but slit-like, and arranged round its margin is a circular band of locomotor cilia. Right opposite the blastopore is the apical thickening, bearing a tuft of hair-like cilia. The growth of the gastrula is not accompanied by elongation in the main axis, but obliquely to this in such a way as to pass through one end of the slit-like blastopore. One end of the blastopore is thus carried backwards away from the other, which remains more or less fixed. The blastopore becomes an elongated slit, the lips of which meet in the middle and close, forming the rudiment of the future gut. For a short time the digestive tract remains attached to the ectoderm throughout the length of the blastopore, but after a little it only retains this connection at either end. With further growth the embryo is converted into the trochophore. The digestive tract becomes hollow and acquires two openings to the exterior at the two points of its previous connection with the ectoderm. That near the ciliated band becomes the mouth, while the other becomes the anus.

"Just before the formation of the anus a number of ectodermal cells near the region of the future anus become separated from the rest of the digestive tract and form a mass of cells lying outside the alimentary canal in the body-cavity. These cells form the mesoderm. Some of these cells increase in size and form stellate mesenchyme cells, and finally a few of them stretch across the body-cavity near the anus, forming a membrane which separates a small portion of the body-cavity from the rest, forming the anal vesicle. Occasionally another partition grows across it, separating it into two smaller divisions." Certain other mesoderm cells form the true mesoderm. "They multiply quite rapidly, and soon give rise to the mesoderm bands. One of the eye-spots develops much before the other" (p. 671).

Von Drasche (**10**), in 1884, gave an account of the development of Pomatoceros, but the early stages and the forma-

tion of the trochophore were very briefly studied. He did not observe the origin of the mesoderm cells.

Hatschek (**17**), in 1885, studied the development of Eupomatus at Trieste. He supplemented these observations by the examination of a small trochophore found in the Pantano, at Faro, Sicily. The identity of this larva he did not definitely establish. The eggs studied at Trieste were fertilised by the addition of ripe sperm, and were studied in the living state. Segmentation is equal, and of the spiral type characteristic of many Polychæts. In the resulting blastula the cells from which the germ layers form are already differentiated. The greater part of the lower hemisphere of the blastula produces endoderm. Two cells here larger than the rest give rise to the primitive mesoderm cells, or teloblasts. The region where they lie corresponds to the anal end of the larva. At this time the pre-oral band of cilia makes its appearance as an equatorial circle of cilia. Shortly afterwards the apical cilia appear. The endodermic part of the blastula invaginates about nine hours after fertilisation. The two mesoderm cells at the same time move to the interior of the segmentation cavity and detach themselves from their connection with the other cells. The invaginated portion of the endoderm forming the gut then bends towards the anal side of the larva, and fuses with a slight depression of the ectoderm and produces the anus and proctodæum. At the same time the blastopore has become narrowed to a slit, which gradually closes from behind forwards. At the place where the last trace of the blastopore remains the ectoderm invaginates and forms the œsophagus. At the same time the two primitive mesoderm cells divide, giving rise to the mesoderm bands, while other cells near the pole-cells of the bands give rise to the head-kidneys; these increase greatly in length and become hollow. The head-kidney then extends from the pole-cells in the region of the anus to the wall of the œsophagus, to which they are attached by a thin protoplasmic strand, while another runs up in the apical region. They open, according to Hatschek, on the exterior on either side of the

anus. The eye-spot is located in a cell in the apical region. There is a peri-anal circle of cilia.

E. B. Wilson (48) briefly studied (1890) the segmentation of the egg of a species of Hydroides found at Naples. The order and direction of the early cleavage planes coincide very closely with those of Eupomatus, and segmentation is of the equal spiral type. The spiral symmetry would seem to be retained until a late stage. He did not definitely observe the cell *4d* or follow its history. In his early paper (48) on the origin of the mesoblast bands of Annelids he was of opinion that the bands gave origin to the mesenchyme cells. He did not observe the pole-cells of the bands as described by Hatschek. He pointed out that the head-kidney probably opened into the proctodæum.

The later development of Psygmobranchus has been studied by Meyer (27), who made some important observations on the mesoderm. He pointed out that in the young trochophore it can be divided under three headings: First, the mesoderm bands, which are closely applied to the ventral surface of the endoderm; secondly, a collection of irregular cells attached to both ecto- and endoderm, which we can call the embryonic mesenchyme; thirdly, a row of functional primary larval muscles. The mesoderm bands appear as a paired plate of cells converging on one another posteriorly, each ending in a pole-cell—the so-called teloblasts. The plates extend forward into the oral region. The cells of the mesoderm bands can be clearly distinguished from the irregular cells of the mesenchyme by their polygonal outlines and their dark-staining nuclei. The larval mesenchyme cells, on the contrary, are irregular in outline, and their nuclei stain less deeply than do those of the bands. The mesenchyme does not form a compact structure, but is somewhat irregularly arranged into masses on the inner wall of the ectoderm or the wall of the gut. It is divided into a median and a lateral portion, which is again divided into a trunk and head portion.

The lateral trunk mesenchyme lies on either side of the

inner surface of the body-wall, and commences behind the teloblast cells of the mesoderm bands, and runs forward in the region of the oral ciliated ring. In the pre-oral region one finds a number of these mesenchyme cells under the body-wall, where they form a portion of the head division of the mesenchyme in relation with the prototroch and apical organ.

The median trunk mesoderm begins behind in front of the anal vesicle, and is continued forward in the median line under the gut into the region of the stomatodæum between the mesoderm bands. The functional larval muscles of the mesenchyme type consist of a ventral and dorsal longitudinal set and the pre-oral circular muscles of the prototroch. With the growth of the larva the greater part of the larval mesenchyme is converted into the definite musculature of the adult. The mesoderm bands in no instance give rise to mesenchyme cells, and the two can be sharply distinguished throughout the course of the larval development.

The development of Spirorbis borealis has been briefly described by Schively (**33**). There is a very small blastocœl, and the blastopore is a median ventral slit. It closes from the posterior end forwards until nothing remains but a small aperture at the anterior end, which becomes the future mouth. The endoderm on invagination forms the archenteron. The mesoderm can be traced to the left posterior macromere, which sinks into the segmentation-cavity, giving rise by a bilateral division to the primitive mesoderm cells. No mention is made of the larval mesoblast.

The early development of Serpula infundibulum has been studied by Soulier (**38**) in 1902. The main outcome of his work has been to confirm very closely Hatschek's results for Eupomatus. The mesoderm cells are recognisable as two large cells in the endoderm at the time of invagination. They arise at the point of union of ecto- and endoderm, and pass into the segmentation-cavity, where they give rise to the mesoderm bands. Their relation to the irregular cells of the mesenchyme was not determined.

Apart from the Serpulids, the development of Thalassema, Podarke, and Polygordius closely resembles that of Eupomatus in its essential features. The cleavage in these is of the equal spiral type of that of Eupomatus, and, in fact, the early cleavage of Podarke, Thalassema, and Eupomatus are almost similar cell for cell until the time of gastrulation. In each the gastrula is formed by invagination, and a well marked blastocœlic cavity is present. In the later stages of gastrulation Thalassema and Polygordius more closely approach Eupomatus than does Podarke. This is possibly due to the fact that the trochophore of Podarke is somewhat modified, apparently not having any head-kidney. Polygordius, with its large blastopore, represents possibly a more primitive condition than do the others. Thalassema in the pre-trochophoral stage approaches nearer Eupomatus than do the others, for in Polygordius the head-kidneys form some time before the mesoderm bands. The details of the resemblance between these four will be considered further on.

3. Material and Methods.

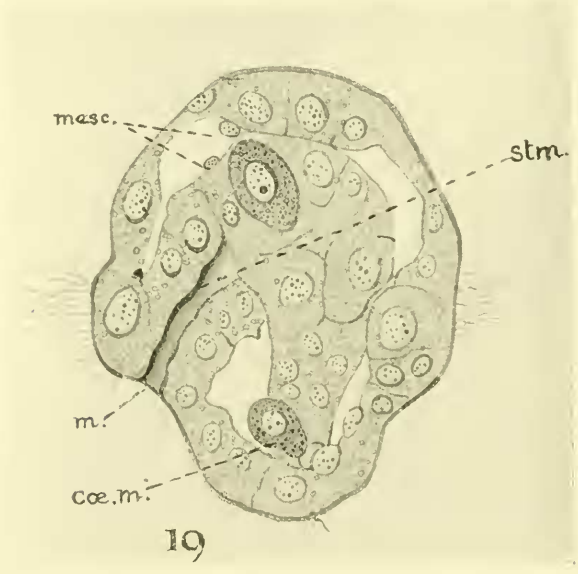
When the sexual products are ripe in Eupomatus it is an easy matter to distinguish the sexes from the colour of the body. The female is bright yellow, while the male is white. In Naples they grow in dense colonies attached to stones, the sexes being evenly proportioned, although the males and females show a slight tendency to occur together in separate spots in the colony. Their tubes stand upright, being attached by one end.

In effecting fertilisation under artificial means, it is unnecessary to wait until the eggs are deposited as in many Annelids, as Nereis, Podarke, Phyllodoce. The ripe eggs cut from the body-cavity fertilise as readily as those laid in the normal manner.

The egg of the Neapolitan Eupomatus seems to be more opaque than that studied by Hatschek at Trieste, for I have

been unable to follow the fate of the invaginated cells during gastrulation in surface views of the living egg as he was able to do. In the following work I have relied entirely on the evidence of sections. By means of the combined celloidin-paraffin method of embedding, one is able to obtain good sections of small gastrulæ such as these. For fixing I have found sublimate acetic and Flemming solution give satis-

Text-fig. 19.

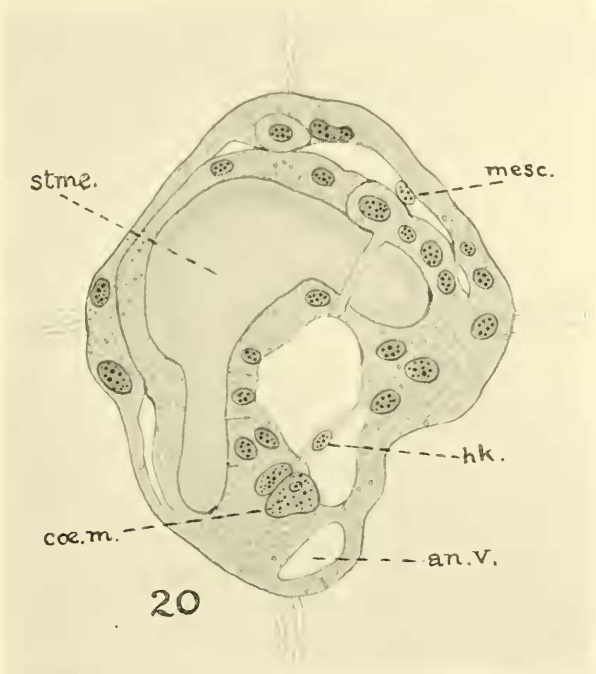


Section of early trochophore of Eupomatus. *coe. m.* Cœlomesoblast. *mesc.* Mesenchyme. *m.* Mouth. *stm.* Stomodæum.

factory results. From these sections I have followed the formation of the gastrula cell by cell. No mistake can be made, therefore, in the position of these cells, as is frequently done in the study of surface views alone, and one does not get flattening and distortion from the pressure of the cover-glass, as is invariably the case in the study of living preparations. The fertilised egg measures about 55 μ in diameter. The eggs laid under normal conditions are almost spherical,

but those obtained from the body-cavity are always flattened and lenticular in shape. On being placed in sea-water, after a short time they fill out and become spherical and regular in outline. They are covered by a thin membrane which remains attached throughout segmentation and gastrulation,

Text-fig. 20.



Section through a trochophore of Eupomatus three days old. *an. v.* Anal vesicle. *cœ. m.* Cœlomesoblast. *hk.* Head-kidney. *stmc.* Stomach.

becoming the cuticle of the trochophore. This in the living egg is smooth and transparent, but shrinks and becomes considerably wrinkled under the reaction of reagents, especially sublimate. This renders the study of fixed material difficult, as the cuticle has a strong affinity for stains, obscuring the underlying cells and adding to the uncertainty of orientation.

This cuticle has been noticed by Stossich (39), Conn (9), and Hatschek (17); the first of these investigators observed that it became the cuticle of the larva. A similar though somewhat thinner membrane surrounds the eggs of Podarke. In Serpula it is even thicker than in Eupomatus, where at the animal pole it leaves quite a space surrounding the polar bodies. A smaller space is found in Eupomatus, in which two dark polar bodies are seen. There is no micropyle, and the sperm seems to be able to penetrate the membrane at any point.

It must be remembered that the type of cleavage of such widely separated forms as Hydroides, Thalassema, Podarke, and Lepidonotus resemble one another on account of their possession of a trochophore. They all possess a free-swimming stage of considerable duration, and as the initial size of the blastomeres stands in direct relation to the size of the part to which they give rise, as pointed out by Lillie (25), the resulting cleavage conforms to the same type.

4. Segmentation and Gastrulation.

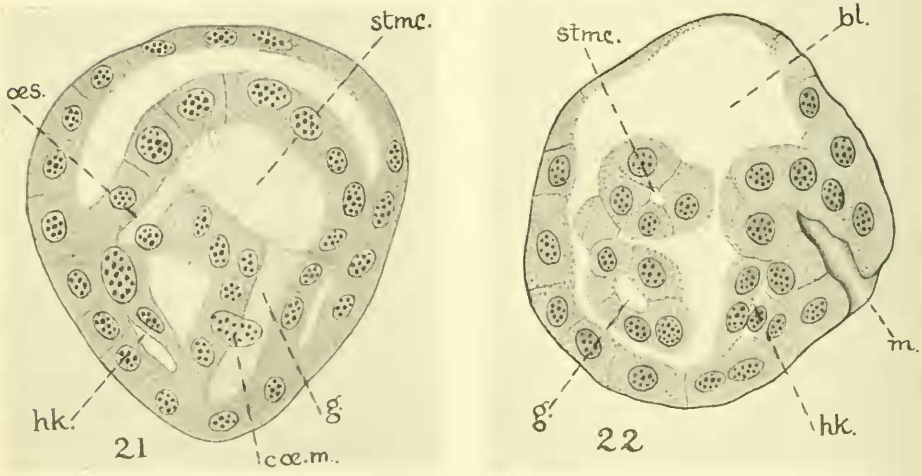
Segmentation begins about one to two hours after the sperm have been added to the eggs. The rate of development naturally varies greatly, being increased with any rise and decreased with any fall in the temperature. With the hot weather in Naples during the summer months, development quickly becomes abnormal unless precautions are taken to keep the water cool in the culture dishes. Segmentation is rapid and regular once it has set in, and results in a blastula containing a segmentation-cavity of variable dimensions. It is of the equal type, and resembles very closely that of the Annelid Podarke, which is remarkable for the fact that the spiral symmetry is retained almost complete up till an unusually late stage. The first cleavage furrow cuts through the egg, sinking in more rapidly at the upper than at the lower pole, and produces the two-cell stage. The first cleavage is

exactly equal; the two nuclei of the cells are opposite one another, and show no tendency to rotate as Conklin (7) has described in Crepidula. The subsequent divisions follow in rapid succession. With the third cleavage four slightly smaller upper cells are separated by a dexiotropic division from the lower macromeres. In the fourth cleavage the micromeres of the second group are of the same size, and are very slightly smaller than the macromeres.

Invagination produces a typical gastrula. Gastrulation usually commences about seven or eight hours after fertilisation, and consists of a sinking in of the ventral ectoblastic plate, all the entomeres of which are alike during the early stage of the process. Gastrulation is of the modified embolic type, with considerable preparatory flattening of the ventral plate. The cells about to sink in elongate, and their nuclei take up a position at their inner swollen ends. While this flattening is taking place the apical portion of the gastrula is rounding out, the apical tuft of cilia commences to appear, and the endoderm cells sink in till they come in contact with the inner wall of the ectoderm, in the region of the "rosette cells." At first there is a complete obliteration of the segmentation-cavity, the endoderm folding up close against the ectoderm; but in the immediate filling out of the gastrula, which takes place almost simultaneously, the ectoderm is again drawn away and the segmentation-cavity reappears (Text-fig. 18). At this stage a number of viscid protoplasmic threads are seen connecting the two layers, and one blastomere with another. They have been observed in Podarke by Treadwell (43), in Serpula by Soulier (38), and in Thalassema by Torrey (41); I have already drawn attention (35) to them in Eupomatus, and pointed out that they are probably similar to the filose strands first described by Andrews (2), and considered by him as cell connections. Prof. Loeb has suggested to me, however, that they are rather more in the nature of the fine cytoplasmic strands so frequently seen in membrane formation during fertilisation than definite cell communications.

The blastopore at first lies exactly in the middle of the ventral plate, and is marked out behind by two large cells, which, as in Nereis, probably belong to the X group (fig. 11). When fully formed it is an elongated slit, somewhat enlarged at its anterior end. This end never completely closes, but after the formation of the stomach becomes the future mouth. The posterior portion closes completely, the anus breaking

TEXT-FIGS. 21 AND 22.



Sections through early trochophores of Eupomatus. *g*. Gut. *œs*. Œsophagus. *m*. Mouth. *hk*. Head kidney. *œ.m*. Cœlomesoblast. *stmc*. Stomach.

through almost immediately at the point where the last portion of this part of the blastopore disappears. Thus the closure of the blastopore in Eupomatus is essentially the same as in Polygordius, although the different steps in the process are not so evident. In the majority of Annelids the blastopore usually closes completely, as in Capitella.

5. THE ECTOMESOBLAST.

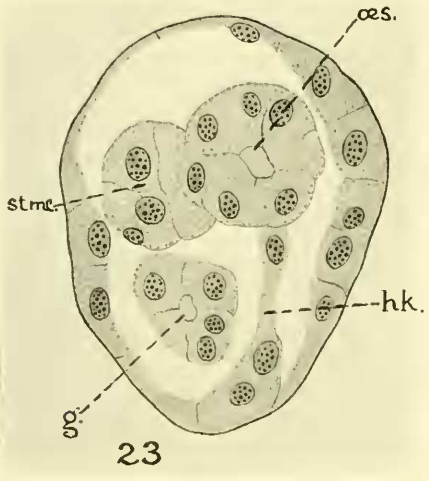
Towards the end of gastrulation some irregular cells are

seen in the segmentation-cavity. Their origin I have not succeeded in observing. They are shown in Text-fig. 18, *1mr.* and *1ml.* I believe they arise from cells of the third quartette, but as I have not followed the cell-lineage carefully, I am by no means certain of their exact origin. They sink into the cleavage-cavity during gastrulation, and take up a bilateral position on either side of the blastopore, as shown in Text-fig. 18. They immediately divide, giving rise to some irregular small cells that apply themselves closely to the wall of the stomodæum, and later form larval muscles. One large cell on either side gives rise to a string of cells, which enter into close relation with the cœlomesoblast. From their mode of origin and their subsequent behaviour I think there can be no doubt that they represent the larval or ectomesoblast of Podarke and Thalassema. In addition to these cells, some mesenchyme cells are also constantly seen in slightly later stages (figs. 9 and 10) in the apical region under the "cross-cells." Whether they arise by migration of some of the cells from the stomodæum, or by the sinking in of ectoderm cells in the apical region, which last I think is more likely, I have not determined. As in Podarke and Thalassema and molluscs, therefore, the larval mesoblast can be divided into the median, the portion under the apical organ, and the right and left portion on either side of the blastopore. These cells (*1mr.* and *1ml.* of Text-fig. 18) would correspond with the right and left parts of the ectomesoblast of Podarke and Thalassema.

It is worth repeating the description of these structures in these forms. In Thalassema, Torrey (41) states, "The most important source of functional mesenchyme, in Thalassema, are the three cells from the third quartette, namely, $3d_{2\,2\,2\,1}$, $3c_{2\,1\,2\,1}$, and and $3a_{2\,2\,2}$. The first two sink into the cleavage-cavity, just before gastrulation, and lie at first close to the cœlomesoblast cells. They soon migrate laterally, and bud off simultaneously small cells towards the mesoblast cells, dividing like teloblasts, but in the reverse of the ordinary direction. So close is the connection of these cells

with the cœlomesoblast (see Text-fig. 9, *1ml.*) that one would certainly be led to think that they formed part of these bands, unless their cytogeny had been carefully followed" (p. 223). They have been described as follows in Podarke by Treadwell (**43**). They arise as in Thalassema from the *3d*, *3c*, and *3a*, and sink into the segmentation-cavity, where they arrange themselves symmetrically, forming bands of three or

Text-fig. 23.



Oblique corneal section through early trochophore of Eupomatus. Lettering as in fig. 22.

more cells. "Since the posterior end of each band lies very close to the definitive mesoblast, the effect is that of a well-developed mesoblast band, lying in the usual position in the segmentation-cavity" (p. 427).

The median portion of the ectomesoblast in Eupomatus retains its position untransformed into larval musculature until a very late stage, when the trochophore becomes segmented. It is shown under the apical organ of the early trochophore in figs. 9, 10, 16 (*mesc.*). In the fully formed trochophore it is shown in figs. 2, 3, and 6 (*mesc.*).

I will now describe in detail the changes undergone by these cells. In a late gastrula stage such as that shown in this text-figure (Text-fig. 18) these cells have already divided; the division usually is an unequal one, in which one of the daughter-cells is much smaller and more irregular in shape than the other. They seldom divide simultaneously on both sides, but the right usually precedes the left. If we refer to fig. 9, we see the larger of these cells attached to the ventral wall of the œsophagus (*hk.*). The smaller seems to give rise to some of the mesenchyme cells that are attached to the wall of the œsophagus. These are very irregular in shape and size. At this early stage they are only seen with difficulty, as they are few in number, and are closely pressed against the surface of the œsophagus. Although the stage represented in fig. 9 has already assumed the shape of the early trochophore, it is but slightly older than the late gastrula stage represented in Text-fig. 18.

The head-kidney strand is derived from the division of the large cell (*hk.*) seen in fig. 9. This divides once, and then by a second division of one of the daughter-cells a band of three cells is formed (Text-fig. 23). The nuclei of these cells so arrange themselves that two remain in the end of the strand attached to the œsophagus, while one moves to the distal end, which abuts against the anal end of the gut. This stage is represented in fig. 12. In fig. 10 the head-kidney cell has divided, forming two daughter-cells, one of which is applied close to the wall of the œsophagus, while the other rests against the inner lower surface of the larval hemisphere. In fig. 12 the two distal nuclei of this band have moved apart, one resting against the anal end of the gut, while the other remains close to the œsophagus.

In Text-figs. 21 and 22 the strand of cells forming the head-kidney is shown in sections. In Text-fig. 21 (*coe. m.*) is shown part of the nucleus of the cœlomesoblast cell. This figure is almost in the median plane, while the plane of section of Text-fig. 22 is quite oblique, showing only a portion of the stomach and gut-wall. The cell boundaries disappear, so

that the head-kidney strand consists of a thin thread of cytoplasm, at either end of which are the nuclei. A fine lumen begins to appear in the middle about the second day; this increases in size and works its way towards either end, and by the middle or end of the third day the organ becomes functional as the head-kidney, having acquired an opening into the proctodæum.

6. The Cœlomesoblast.

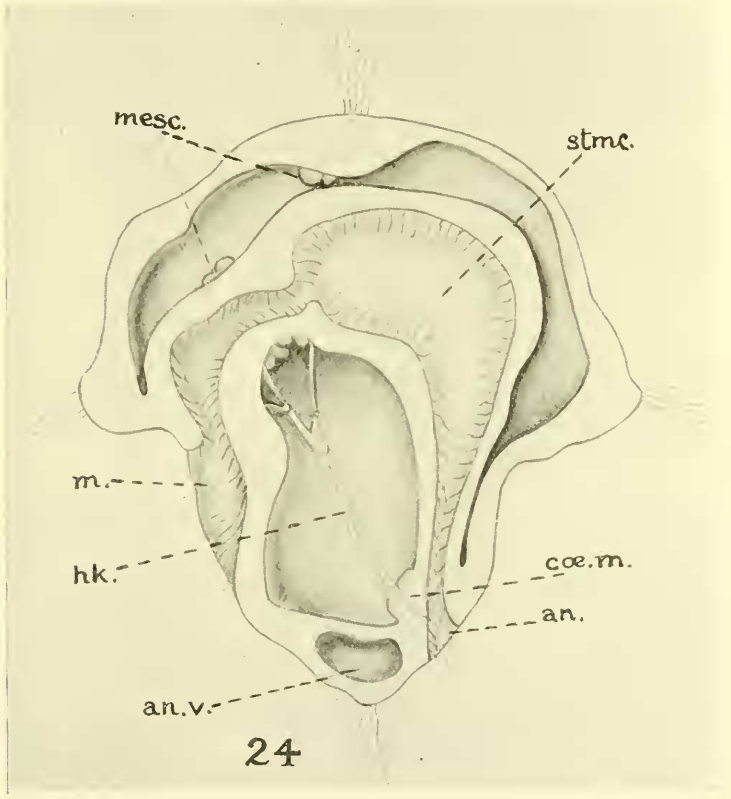
Towards the end of gastrulation, and after the period when the ectomesoblast has already appeared in the blastocœl, two large cells are seen side by side in the ventral lip of the blastopore. In surface views they seem to lie more in the ventral ectodermic plate than in the endoderm. From sections, however (Text-fig. 17, *me.*), they are seen to be part of the endoderm at its point of junction with the ventral plate. They are not free in the segmentation-cavity, and during the course of invagination they come to lie in the wall of the primitive archenteron. They finally rest in the anal end of this structure, where, at much later stages, by a series of rapid divisions, they give rise to a number of cells which push out into the blastocœl and form the mesoderm bands. They are, therefore, the cœlomesoblast cells.

In the stage represented in section in Text-fig. 17 they are usually seen in the ventral lip of the blastopore undergoing division. The fate of the smaller of the resulting daughter-cells I have been unable to determine, but I believe they represent the small cells forming part of the wall of the archenteron in Podarke. The larger of the two cells becomes the cœlomesoblast. As development advances they are carried back in the wall of the archenteron, and do not lie free in the blastocœl till a later stage. In late stages they are seen in the anal end of the archenteron as in Text-figs. 19–25 (*cœ. m.*); here they always project slightly from the gut-wall.

After their division, as shown in Text-fig. 17, the various

steps by which the larger of the two cells is shifted back into the anal region are somewhat difficult to follow. Sometimes they do not appear to differ greatly from the surround-

TEXT-FIG. 24.



Diagrammatic figure of an early trochophore of Eupomatus before the formation of the mesoblast bands, and showing the opening of the head-kidney into the proctodæum. *cœ.m.* Cœlomesoblast. *an.* Anus. *an.v.* Anal vesicle. *mesc.* Mesenchyme or ectomesoblast. *hk.* Head kidney. *m.* Mouth. *stmc.* Stomach.

ing cells, but they can usually be distinguished by their greater affinity for stains; and in late stages they can always be recognised by the way they are wedged into the

gut wall above the anal vesicle. Close examination of the sections shows them first as two cells in the ventral wall of the stomach, and then the gut. The change that has to do mostly with bringing this about is the great increase in the dorsal surface of the gastrula and the consequent narrowing of the blastoporal surface, changing the large ventral to a small ventro-lateral surface. At a time when the anal opening of the gut has not been established they occupy about the mid-region of the archenteron. At the period when the anus breaks through they have already moved into the anal end.

The blastocœl during this time is still small, and has not undergone the great increase it shows shortly after this period, as only a trace of it can be seen between the gut and the ectoderm. This adds somewhat to the difficulty of determining how the various steps in the process take place. The primitive trochophore about this time begins to assume its typical shape ; up to this the round shape of the gastrula has been retained. During early gastrulation before the division of the cœlomesoblast cell, as shown in Text-fig. 17, I have been quite unable to distinguish it from any of the other endoderm cells. No conspicuous cell is seen forcing its way into the segmentation-cavity as shown by Hatschek (**17**) and Soulier (**38**), and I believe that both these investigators have been mistaken in their identification of the cœlomesoblast cell. The cell shown in Hatschek's figs. 25–36, and in Soulier's figs. 25–27 and 33 and 34, and identified by them as the cœlomesoblast, are really the right and left portions of the ectomesoblast. At a later stage they give rise to the head-kidneys. The real cœlomesoblast at this period still lies in the gut-wall, and not free in the blastocœl.

In the late gastrula stages the right and left portions of the larval mesoblast appear as shown in Text-fig. 18. In all respects these cells answer to the mesoderm cells of Hatschek's figs. 25–37, fig. 9 of this paper corresponding to Hatschek's fig. 33. By a comparison of figs. 9, 10, 12, 13, 15, 16, the various changes will be seen by which these cells are transformed almost entirely into the head-kidneys. In fig. 16 the

mesoblast bands have not appeared. In the young trochophore shown in fig. 1 they are just appearing as they grow out from the gut-wall. As these cells just mentioned are converted into the head-kidneys before the mesoblast bands have appeared, it is fair to assume that they do not represent the cœlomesoblast cells as Hatschek and Soulier claim. It must also be recalled that both these investigators have not followed the cell lineage, and therefore they have no definite grounds of cytological importance on which to substantiate their claim as to the nature of these cells.

In the early stages of invagination it is certain that the cœlomesoblast cells cannot be distinguished, as these investigators state, by their conspicuous size and the manner in which they force their way into the segmentation-cavity. In fact, I have been only able to distinguish them satisfactorily in early stages by following their development backwards from a stage when these are clearly recognisable in the anal end of the gut to a stage towards the end of gastrulation; prior to this I cannot see that they differ from any of the other endoderm cells.

In fact, the condition in Eupomatus is simply a more marked type of that found in Podarke. In this Annelid, according to Treadwell (**43**), at the sixty-four-cell stage the fourth group of micromeres have just formed. They are all alike, but shortly one of them divides bilaterally, thus aiding substantially in the establishment of the bilateral symmetry. Then each buds off a small cell ventrally; these small cells form a part of the wall of the archenteron. During the course of gastrulation the mesoblast cells lie in the wall of the archenteron, with which they are carried inwards, finally coming to lie in the anal region. They protrude considerably, and in sections that pass (Text-fig. 6A) a little to one side of the sagittal plane they seem to lie actually free in the segmentation-cavity.

The condition in Podarke and Eupomatus, again, is only a more marked state of that found in Crepidula, where the greater portion of the primary mesoblast cell

contains endoderm, remaining a mesendoblastic cell for eight divisions before the mesoblastic is separated from the endodermic portion. The endodermic part enters into the wall of the archenteron. In Eupomatus and Podarke the greater part of the mesoblast cell (*4d*) is mesoblastic, while in Crepidula only a small part of it is mesoblastic. In Amphitrite, Mead (**26**) represents the mesoderm cell similarly giving off a small cell. The spindle of this division, as in Eupomatus, lies in the short diameter of the cell, which at this moment is compressed between the ventral wall of the ectoderm and the main mass of the invaginated endoderm. The axis of the spindle is in the direction of greatest pressure.

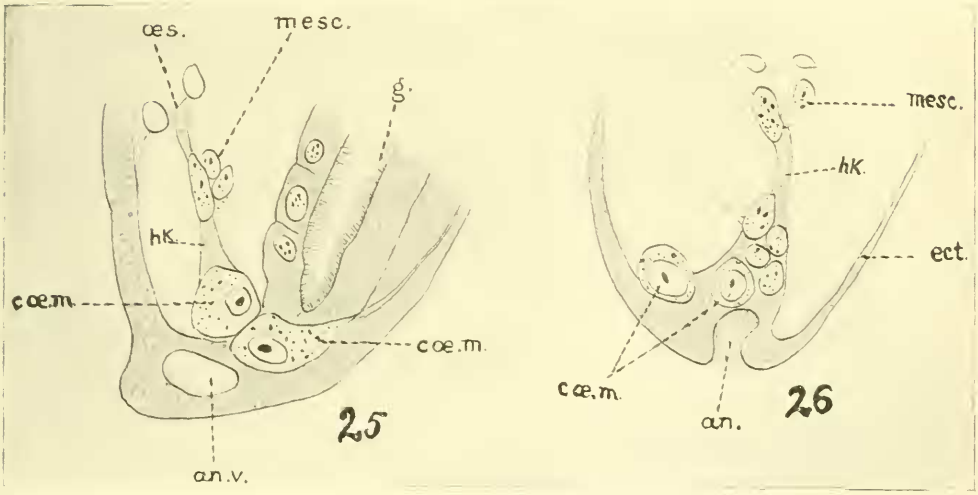
In Thalassema the mesoblast cells, which at first are pressed together under the ventral lip of the blastopore, separate and move apart towards the sides, lying well up towards the prototroch (Text-fig. 9, *me.*). As they move apart they divide rapidly, each giving rise to a group of five or more cells, which form the mesoblast bands as in Annelids. They are quite free in the blastocœl, and enter into close relation with the right and left portions of the larval mesoblast, from which they can be distinguished, as in Eupomatus, by their different staining reaction. Thus Thalassema represents a condition midway between that of Eupomatus and Physa and other Molluscs, where the mesoderm cell lies free in the blastocœl from the time of invagination.

In Polygordius I have shown (**37**) that the head-kidneys form early and before the mesoderm bands have appeared; that the rudiments of these organs are first recognisable as two cells in the ventral plate of the ectoderm. They grow out into the blastocœl, and by division give rise to a string of cells, as in Eupomatus, that run up to the œsophagus. They fuse together and become one strand of cytoplasm, with three or more nuclei. This then hollows out, develops a flagellum, and becomes functional as a head-kidney, at an age when the mesoderm bands are represented by a few cells on either side of the anal opening.

I have advanced reasons for believing that the head-kidney

strands in Polygordius are in many ways comparable to the lateral portions of the larval or ectomesoblast of Thalassema and Annelids. The condition in Polygordius, where the ectomesoblast arises and becomes functional so much earlier than the cœlomesoblast, shows that the head-kidney strands do not form from the bands, and this point is borne out by the cell-lineage as worked out by Woltereck (52). In Eupomatus the formation of the cœlomesoblast follows so

TEXT-FIGS. 25 AND 26.



Sections through the anal ends of early larvæ of Eupomatus. *an.* Anus. *an.v.* Anal vesicle. *coe.m.* Cœlomesoblast. *hk.* Head kidney. *ect.* Ectoderm. *g.* Gut. *œs.* Œsophagus.

closely on that of the ectomesoblast that this difference is not so marked.

To sum up: the cœlomesoblast in Eupomatus is not recognisable until a relatively late stage in gastrulation, and the cells described by Hatschek and Soulier as the mesoderm cells are probably portions of the larval or ectomesoblast. At the time the ectomesoblast is represented by two cells on each side of the mouth, the cœlomesoblast is represented by a cell in either side of the gut-wall above

the proctodæum. Only in the trochophoral stage does the cœlomesoblast divide, giving rise to the mesoblast bands, which gradually grow up the head-kidney ducts to the region of the œsophagus. There is relatively a considerable period during the trochophoral stage, when the larva is without mesoderm bands, and the rudiments of the bands are represented by a single cell on either side of the gut-wall in the anal region.

7. The Early Trochophoral Stages.

In part the early trochophoral stages have been considered in the foregoing section. Before the completion of gastrulation the larva begins to assume the shape of the trochophore. Figs. 9, 10, 12, 13, 15 and 16 show the shape of the early larvæ; of these probably fig. 12 is the most typical. In these figures the upper and lower larval hemisphere is dome-shaped and rounded, as compared with the pointed and more conical appearance of the mature larvæ shown in figs. 1, 2, and 3. The apical cilia, cilia of the mouth, prototroch and paratroch, are, for the sake of simplicity, not shown in these figures, which are drawn from fixed material, and are therefore more granular looking than the living larvæ. These stages are derived from the gastrula about the twentieth to the thirtieth hour of development. At this time there is a great thinning out of the tissues, and the larva rapidly increases in size. In the region of the prototroch a very active proliferation of the cells is taking place, by which the gastrula is lengthened out into the conical dome-shaped larva. The primitive archenteron becomes sharply divided into the cylindrical œsophagus, cubical stomach, and narrow gut. The cells of its walls are seen dividing rapidly. The inner surface of the œsophagus secretes a cuticle, as in Thalassema (Text-fig. 19). The archenteron is lined throughout with strong cilia. Those of the œsophagus are remarkably long and powerful. The inner wall of the stomach is covered uniformly with fine cilia, which keep the food contents in constant motion. The cilia of the

gut are somewhat longer and more powerful than those of the stomach. Immediately above the proctodæum the lumen of the gut is narrowed down by a projecting ridge. This is well shown in the trochophore of Hydroides pectinata (fig. 18). Below this constriction the gut opens into the proctodæum, which, like the stomodæum, also secretes a fine cuticle. At first the cells of the archenteron are uniformly cubical in appearance, but those of the œsophagus and the gut soon thin out, while those of the stomach alone retain their primitive appearance.

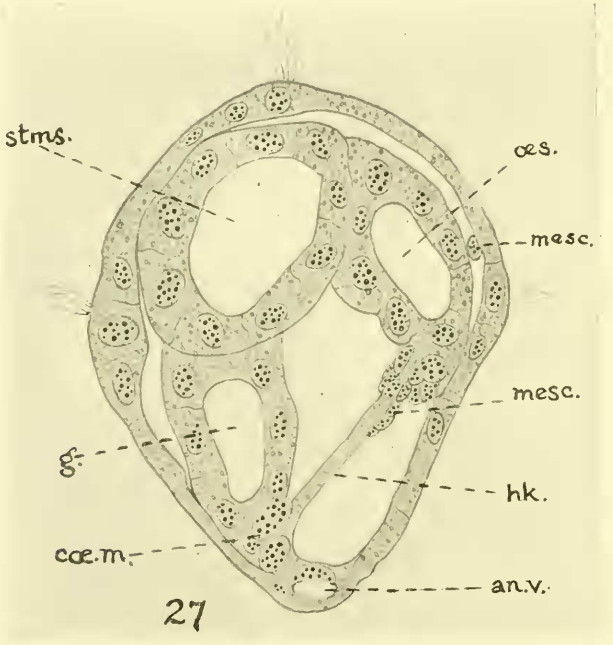
The larva at this time has the shape represented in figs. 9, 10, and 12. The anal vesicle begins to appear as a small vacuole in one of the ectoderm cells of the anal region. This at first connects with the exterior by a small duct, but this soon closes, and the vesicle increases rapidly in size. The cytoplasm of the cell stretches so that a thin envelope alone is left which surrounds the vesicle. It then becomes constricted into two portions, as shown in fig. 14. The original nucleus of the cell is seen projecting into the blastocœl from the upper wall of the vesicle.

On either side of the gut, just above the anal vesicle, a large conspicuous nucleus is seen embedded in the wall. This is the nucleus of the cœlomesoblast cell. In the stages represented in figs. 9 and 10 it is not so prominent as in the later stages shown in figs. 12, 13, 15, and 16. As development proceeds it is pushed out more and more into the blastocœl. In Text-fig. 19 it appears to be free in the blastocœl, but examination of the subsequent sections of this series clearly shows it to lie in the gut-wall. As I have mentioned, it is of somewhat different staining reaction to the surrounding cells, and this contrast is shown somewhat in this text-figure, which is from a camera drawing of an actual section. The section passes a little to one side of the median line, and is slightly oblique, as the mouth and œsophagus are cut in the median plane, while the section passes through the lateral wall of the stomach and the gut. In Text-fig. 20 is shown a section of an older stage in which

the head-kidney has formed, and the mesoderm cell is seen wedged in between the anal vesicle and the head-kidney.

The growth of the bands from these cells is not that of a true teloblastic one; when the cœlomesoblast cells start to divide they do so quite irregularly. The bands at first consist

TEXT-FIG. 27.



Section through trochophore of Eupomatus older than those of the foregoing figures. *an.v.* Anal vesicle. *cœ.m.* Cœlomesoblast. *g.* Gut. *hk.* Head kidney. *mesc.* Mesenchyme or ectomesoblast. *stms.* Stomach.

of groups of three or four cells; they divide in all directions, so that after the first division it is not possible to speak of a pole-cell, the divisions always being equal. Hatschek's rather elaborate account of the origin of the bands by teloblastic growth conveys quite an erroneous impression of the process. The cœlomesoblast cell first divides into two equal cells, and

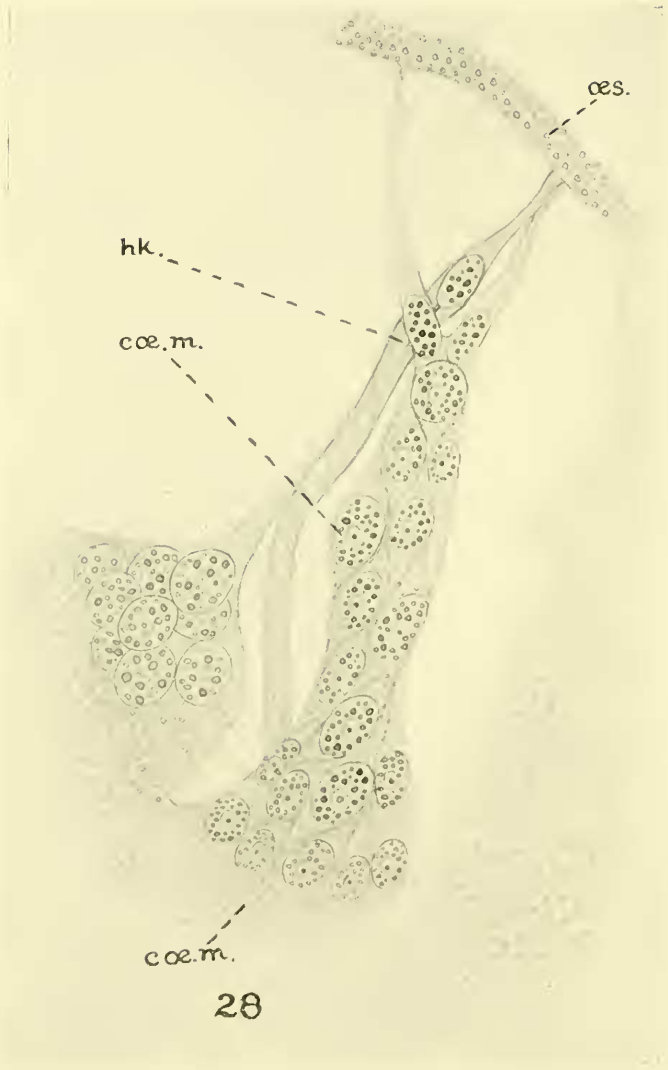
these, again, in turn divide equally. One cell remains attached to the gut-wall, as shown in a late stage in fig. 17 (*pm.*), but this cell does not divide in this stage, and the growing point of the bands is not here, but towards the ends nearest the œsophagus. The position of this cell alone gives it the appearance of being a pole-cell.

According to Wilson (48) there is a complete absence of teloblastic growth in the species of Hydroides studied by him, for he states: "I have carefully studied the development of Hydroides dianthus (a form nearly allied to Eupomatus) by following the cleavage of the living ovum, by examination of stained and cleared embryos, and actual sections. The cleavage is in every detail identical with that of Eupomatus, the gastrulation takes place in essentially the same manner, and the trochophore is of quite the same type. Yet I have been unable to identify the teloblasts at any period. They are certainly not present at a stage when the mesoblast bands consist of not more than four or five cells each. At this period each band ends posteriorly in a group of about three cells, two of which are not perceptibly larger than the others, are joined by a narrow bridge of protoplasm stretching across in the angle between the proctodæum and the wall of the anal vesicle" (p. 215).

In Thalassema, Torrey (41) has not been able to find a teloblastic growth of the bands. "It is a fact," he says, "as far as I know, without exception, that in all forms where there is a trochophore stage of long duration (as in the case of all Annelids with equal cleavage), the two cœlomesoblast cells do not, in the earlier stages at least, bud like teloblasts" (p. 222).

As the bands grow out from the gut-wall in Eupomatus, they keep quite apart from the mesenchyme cells of the blastocœl, nor have I been able in any of the stages I have studied to observe the origin of these cells from the ends of the bands. This is a very debated point in Annelid embryology. Are not the numerous mesenchyme cells of the blastocœl in part derived from the ends of the bands? So

Text-fig. 28.



Head-kidneys and mesoblast-bands in a late larva of Enpomatus.
cœ.m. Cœlomesoblast. *hk.* Head kidney. *œs.* Œsophagus.

recent an investigator as Treadwell (43) is of opinion that they have such an origin. He, however, did not trace the bands in Podarke beyond a stage when they were represented by a few cells, so he obtained no definite information on this point.

In Eupomatus it is clear that the anterior ends of the bands never give off cells into the blastocœl as Hatschek has described. They can be plainly observed throughout the course of their growth; they are always a compact mass of cells, clearly distinct from the larval mesenchyme. The larval mesenchyme cells enter into close relation with the cells of the bands, as may be seen in Text-figs. 25 and 26, and in part overgrow them, but even in the living condition they can usually be distinguished. In sections in which the fixation has been rapid they can readily be separated on account of their different staining properties—a point that has been extensively used by Meyer in his numerous studies on this question.

In Eupomatus a large part of the larval musculature has already been laid down before the formation of the bands has taken place, the greater part of this musculature persisting and ultimately forming a very considerable portion of the adult body.

Meyer (27) has criticised Hatschek's statement regarding the origin of the mesenchyme in Eupomatus, and has expressed himself as being very sceptical as to whether cells arise from the anterior ends of the bands. He is of opinion that, with more modern technique than that employed by Hatschek, whose observations were restricted to living material and optical sections, the facts of the case will prove different. He points out that while Hatschek describes the cœlomesoblast pole-cells as giving off cells into the blastocœl before they form the bands, he neither figures nor appears to have seen the division of these cells. Torrey (41), in speaking of the resemblance of the ectomesoblast in Podarke and Thalassema, says, "The striking similarity in the origin of the ectomesoblast in these two forms justifies us, I believe, in supposing that we may have the same condition of affairs in Eupomatus where the cleavage is also equal" (p. 226).

From Text-figs. 27, 28, which represent sections through the growing bands and head-kidneys of the four-day trochophore, it will be seen that there are numerous mesenchyme cells about the head-kidneys which could hardly have arisen from the cœlomesoblast cells (*cœ.m.*), which, moreover, show no evidence of having recently divided. I have examined a large number of such sections without observing in a single instance the division of these cells to form mesenchyme.

Treadwell (**43**) holds the view that this separation of the mesoderm in Annelids into apparently distinct portions is only a mechanical result of development, but the varied conditions under which a larval mesenchyme is present in Annelids seems to me to be against this view. Treadwell (**43**) has pointed out that we are forced to believe in two non-homologous sets of larval mesenchyme, the one arising from the ectoderm as in Thalassema and Podarke, and the other from the anterior ends of the germ-bands, as in Nereis and Lumbricus. These two sets do not, as a rule, exist together. "On the other hand," he says, "no one has proved, as far as I know, that no 'mesenchyme' arises from the germ-bands in cases where a larval mesenchyme exists." I have attempted to show that in Eupomatus, where a larval mesenchyme exists, no evidences of its origin from the bands can be observed, and the main result of my work has been to emphasise the distinction between ecto- and cœlomesoblast. I have already considered in the "Introduction" whether we are justified in laying any stress on this point. In Annelids we are at least certain that this separation seems general and definite.

Summary.

Segmentation results in a round blastula with a very reduced blastocele. Invagination produces at first an almost spherical gastrula. But this soon begins to assume the conical shape of the early trochophore. The blastopore, which is

small, closes from behind forwards, the anterior portion remaining as the mouth, while the posterior closes completely, the anus breaking through immediately at this point. The blastopore, which was originally ventral, becomes shifted to a ventro-lateral position. At a time when gastrulation is about half completed, some cells appear on either side of the endoderm and take up a bilateral position. They probably correspond to the lateral portions of the larval or ectomesoblast of Thalassema. They subsequently form the head-kidneys in Eupomatus. At the same time two conspicuous cells are usually distinguishable in the ventral lip of the blastopore. These are the cœlomesoblast cells. In the further progress of invagination, they are carried inwards in the wall of the archenteron, finally coming to lie in the anal end of the gut. Here at a considerably later stage they give rise to the mesoderm bands. There is a short stage in the early trochophore when the head-kidneys are already functional while the mesoderm bands are alone represented by these two cells in the gut-wall. With the formation of the bands the organisation of the trochophore is completed. The bands during their growth are never seen to bud off cells into the blastocœl. They remain from the first a compact mass of cells clearly distinguishable from the irregular cells of the ectomesoblast and the head-kidneys. The head-kidneys open into the proctodæum. They are formed from the ectomesoblast.

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EXPLANATION OF PLATES 21–23.

Illustrating Mr. Cresswell Shearer's paper "On the Development and Structure of the Trochophore of Hydroides uncinatus (Eupomatus)."

Lettering.

œs. Œsophagus. *an.* Anus. *an. v.* Anal vesicle. *ap. s.* Apical muscle-strand. *bl.* Blastopore. *cœ. m.* Cœlomesoblast. *eo.* Otocyst. *e.* Eye spot. *ect.* Ectoderm. *end.* Endoderm. *g.* Gut. *hk.* Head-kidney. *m.* Mouth. *mesc.* Mesenchyme or ectomesoblast. *oc.* Otocyst. *seg. c.* Segmentation cavity or blastocœl. *Stm.* Stomach.

PLATE 21.

Fig. 1.—Fully-grown free-swimming trochophore of Eupomatus three days old. The mesoderm bands are just commencing to appear. The head-kidney is shown opening into the proctodæum while the closed end is attached to the œsophagus. This and the subsequent figs. 3, 7 and 8 are drawn from living larvæ compressed slightly under a cover-glass.

Fig. 2.—Trochophore of Hydroides norvegica.

Fig. 3.—Trochophore of Eupomatus four days old, showing the otocyst and mesoderm bands well formed.

Fig. 4.—Head-kidney in a three-day old larva of Eupomatus.

Fig. 5.—Head-kidney in larva of Hydroides norvegica.

Fig. 6.—Trochophore of an unknown Annelid (probably Hydroides pectinata) from an outline drawing by Professor E. B. Wilson, showing the opening of the head-kidney into the proctodæum.

Fig. 7.—Trochophore of Eupomatus three days old. Seen from the ventral surface, showing the junction of the head-kidney on one side with the gut.

Fig. 8.—Trochophore of Eupomatus three days old seen from the oral side. The head-kidneys are shown on either side running down to open into the proctodæum.

PLATE 22.

Fig. 9.—Whole preparation of a larva of Eupomatus twenty-four hours old. In this and in the subsequent figures of this plate the cilia

on the external surface are not shown, for the sake of clearness. The head-kidney cell is seen on the ventral side of the œsophagus. In the apical region some ectomesoblast cells are shown.

Fig. 10.—Slightly older stage than that of the last figure. The head-kidney is represented by a string of three cells.

Fig. 11.—External view of a late gastrula of Eupomatus showing the portion of the blastopore that remains as part of the mouth.

Fig. 12.—Still later stage than that shown in fig. 10. This stage is about thirty-six hours old.

Fig. 13.—Still later stage than the last.

Fig. 14.—Anal end of a young trochophore of Eupomatus showing the double formation of the anal vesicle.

Fig. 15.—Early trochophore of Eupomatus older than that of fig. 13.

Fig. 16.—Early trochophore of Eupomatus forty-eight hours old. The cœlomesoblast cell is seen in the wall of the gut above the anal aperture.

PLATE 23.

Fig. 17.—Hydroides norvegica. The trochophore in this figure is represented as tilted up and seen from the oral surface. The cœlomesoblast is seen arising from two cells in the gut-wall dorsal to the anal vesicle.

Fig. 18.—Hydroides norvegica. The lower portion of the trochophore is shown under high magnification and slightly compressed under the cover-glass. The opening of the head-kidneys into the proctodæum is shown, and the cœlomesoblast.